ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 12 | December - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A COMPARATIVE STUDY OF EFFECT OF TWO PROTOCOLS OF GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) ON THIN ENDOMETRIUM IN FROZEN THAWED EMBRYO TRANSFER CYCLES AT A TERTIARY CARE IVF CENTRE OF RAJASTHAN

Medicine

Dr Anita Sharma Professor & Head, Department of Reproductive Medicine and Surgery, SMS Medical College and Hospital, Jaipur.

Dr Bhanuja Choudhary Assistant Professor, Department of Reproductive Medicine and Surgery, SMS Medical College and Hospital, Jaipur.

Dr Banita Yadav Junior Resident , Department of Obstetrics and Gynaecology, SMS Medical College and Hospital, Jaipur.

Dr Samiksha Department of Reproductive Medicine and Surgery, SMS Medical College and Hospital, Jaipur.

Dr Mamta Meena* Associate Professor, Department of Reproductive Medicine and Surgery, SMS Medical College and Hospital, Jaipur. *Corresponding Author

ABSTRACT

Introduction: Embryo quality and endometrial receptivity are widely recognized as a major determinant of ART outcomes, particularly in frozen–thawed embryo transfer (FET) cycles .It is estimated that implantation failure is responsible for approximately 50% to 75% of lost pregnancies.[1] Endometrial thickness (EMT) is the most widely used prognostic indicator for measuring endometrial receptivity .An endometrial thickness of <7 mm is considered suboptimal for embryo transfer and has a negative effect on pregnancy rate. Several modalities of treatment has been tried in patients with thin endometrium to improve endometrial thickness and receptivity like Tamoxifen ,letrozole , HCG, Vasoactive agents and platelet rich plasma (PRP). In this study, we investigated the role of granulocyte colony-stimulating factor (G-CSF) in improving endometrial thickness in FET Cycles. **Material & Methods:** This interventional prospective study included 90 patients undergoing FET cycles with persistent thin endometrium (<7mm) despite maximum Estradiol dosage. Study population was divided into 2 groups of 45 women each. In Group- A , Injection G-CSF was administered subcutaneously while in Group-B, G-CSF was instilled intrauterine . Both groups were compared in terms of increase in endometrial thickness, vascularity , change in pattern , implantation rate and clinical pregnancy rate. **Results:** Mean endometrial thickness increased in both groups, with a significantly greater increase in Group B. The Implantation rate was 26.6% in Group A and 40% in Group B . No significant change seen in pattern of endometrium were observed in either group. **Conclusion:** G-CSF was effective in improving thin endometrium in FET cycles , with intrauterine route demonstrating superior outcomes compared to subcutaneous route. Multi-centric studies with larger sample size are recommended to establish definitive treatment protocol.

KEYWORDS

In vitro fertilization (IVF), Frozen-thawed embryo transfer cycles (FET), Human chorionic gonadotrophin (hCG), Granulocyte colony stimulating factor (G-CSF)

INTRODUCTION:

Assisted reproductive technology (ART) outcomes are influenced by both embryo quality and endometrial receptivity, with implantation failure being a major limiting factor . Approximately 50-70% of lost pregnancies are attributed to implantation failure [1]. The improvement of cryopreservation-techniques in ART & improved survival rates of blastocyst stage embryos have led to a increase in number of frozen-thawed embryo transfer cycles (FET) in comparison to fresh embryo transfer. Persistent thin endometrium in FET cycles is both challenging and frustrating for clinician and patients. It can lead to repeated cycle cancellations causing monetary loss and increased time to pregnancy interval.

Thin endometrium is often defined by cut-off value of 7 mm.[3] Thin endometrium is considered <7 mm on the day of ovulation, or on the day of human chorionic gonadotrophin (HCG) injection in fresh in vitro fertilization (IVF) cycles, or the day to start progesterone in frozen-thawed embryo transfer cycles.[4] Implantation is a process dependent on three main factors good quality embryos, receptive endometrium with good endometrial thickness and the endometrium-embryonal cross dialogue. Hence implantation of a mature embryo into receptive endometrium is key to build a successful pregnancy. In addition to embryo quality, the receptivity of the endometrium plays a main role in clinical outcome.[5]. Endometrial thickness(EMT) is an important component of endometrial receptivity.

Various strategies have been employed to improve EMT , including hormonal management by estradiol ,tamoxifen, letrozole, HCG and gonadotropin releasing hormone ,vasoactive agent such as low-dose aspirin, high-dose estrogen, transvaginal sildenafil citrate, nitroglycerin and treatment with pentoxifylline and tocopherol[2]. Despite these interventions , a subset of patients continues to have thin endometrium , necessitating novel therapeutic approaches .Other therapies like Intrauterine infusion of growth factor such as G-CSF and the platelet rich plasma are promising.

Granulocyte colony-stimulating factor (G-CSF), a glycoprotein cytokine, has emerged as a potential treatment for thin endometrium. G-CSF stimulates proliferation, differentiation, and migration of hematopoietic cells, and has been shown to promote endometrial growth and vascularization. Clinical studies suggest that both intrauterine and subcutaneous administration of G-CSF may enhance endometrial thickness , improve vascularity, and increase implantation rates in FET cycles.

The present study aims to compare the effectiveness of two protocols of G-CSF-subcutaneously versus intrauterine administration- in patients with persistent thin endometrium undergoing FET cycles, assessing changes in EMT, endometrial pattern, vascularity implantation and clinical pregnancy rates.

MATERIAL AND METHODS:

This Prospective interventional study was conducted in the IVF Centre of Department of Obstetrics and Gynaecology , SMS Medical College, Jaipur from April 2021 to February 2022. Ethical approval was obtained from Institutional ethical committee, and written informed consent was obtained from all participants prior to enrollment. A total of 90 women with persistent thin endometrium (<7 mm) unresponsive to other treatments and undergoing frozen embryo transfer cycles were included in this study.

Sample size calculated at 80% study power and alpha error of 0.05 assuming standard deviation of 2.4 in endometrial thickness as found in the seed article.[6]

Inclusion Criteria

1. Age between 21-40 years of age.
2. Poor endometrial response (endometrium thickness <7 mm) to standard hormonal replacement therapy on the 14th day of FET cycle.
3. Have at least 2 frozen grade1 day 5 embryo.
4. Women willing to participate with informed and written consent.
5. Women not participating in any other study.

Exclusion Criteria:

1. Uterine anomaly.
2. Endometrial polyp and sub mucosal myoma.
3. Asherman syndrome.
4. Uterine Tuberculosis
5. Any intrauterine pathology in hysteroscopy

Randomization and Study groups- All eligible women fulfilling inclusion criteria were explained about the nature and purpose of study and were randomly assigned into two groups :-
GROUP- A (Subcutaneous G-CSF)
GROUP- B (Intrauterine G-CSF)

Endometrial Preparation- all patients underwent standard endometrial preparation for FET, and were started on tablet Estradiol Valerate in a dose of 6 to 8 mg/day from day 2 of cycle after a baseline ultrasound. This was gradually increased upto 12 mg/day in divided doses as needed.

After evacuation of urinary bladder Serial Transvaginal Ultrasound examinations were done using transvaginal probe, by Sonoscope SSI 6000 digital color Doppler ultrasound system, starting from day 7/8, and repeated as required. Endometrial thickness was measured in the median longitudinal plane of the uterus, as the maximum distance from one basal endometrium interface across the endometrial canal to the opposite endo-myometrial interface. Endometrial vascularization was assessed on longitudinal scan of the uterus.

Group-A: Inj G-CSF 300 mcg were given subcutaneously on day 14, endometrial thickness was reassessed after 48 hour by USG, if endometrial thickness <7 mm, G-CSF was repeated again.

Group-B: Inj G-CSF 300 mcg instilled intrauterine on day 14, endometrial thickness was reassessed after 48 hours by USG. If endometrial thickness was found to be less than 7 mm, a second infusion ofG-CSF was performed.

No adverse effects related to G-CSF were observed during the study.

Patients were asked to continue the estradiol preparation in the same dose as before. Repeat ultrasounds were done 48 hrs later, by the same observer and the endometrial thickness, pattern and vascularity were noted. Patients who achieved a satisfactory endometrium (endometrial thickness of > 7 mm with moderate to excellent vascularity pattern)were started on intramuscular progesterone 100 mg daily.

Single Embryo transfer was done on day 5 of progesterone with standard technique. Appropriate luteal phase support was provided and serum beta HCG was measured 2 weeks later. Patient with beta HCG>50 mcg were considered as positive pregnancy. In these patients luteal phase support was continued and transvaginal ultrasound was done 2 weeks later for confirmation of fetal cardiac pulsation (clinical pregnancy).

Outcome Measures:
Primary outcome- change in endometrial thickness (EMT) from baseline to pre transfer measurement.

Secondary outcome – Endometrial pattern (trilaminar versus homogenous) ,Endometrial vascularity (assessed by doppler ultrasound) , chemical pregnancy (serum beta hCG titer >50 mIU/ml) and clinical pregnancy defined as the presence of an intrauterine gestational sac on TVS at 6 weeks.

Statistical Analysis
Statistical analysis was done using data analysis software system, Statistical Package for Social Sciences (SPSS Version 20). Continuous data were expressed as mean ± SD. Paired t test and Wilcoxon rank sum test were applied for the continuous data. $P < 0.05$ was considered statistically significant.

RESULTS:
Out of 90 women, 45 were assigned to group A (subcutaneous CSF injection) and 45 to group B(intrauterine CSF injection). Baseline characteristics were similar in both the groups.

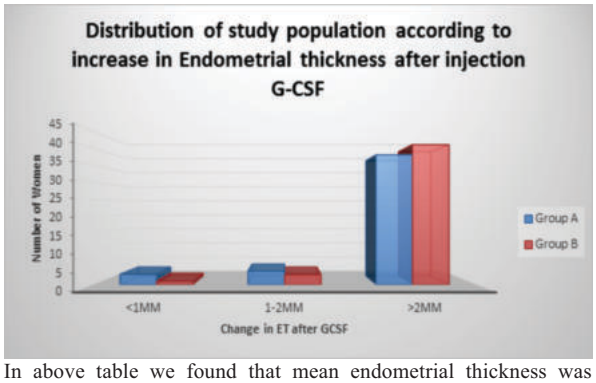
In below table we found that mean age for group A was 31.7 years and for group B it was 30.8 years.

Table 1: Distribution Of Study Population According To Age

Age distribution (years)	Group A		Group B		P-value
	No. of women	Percentage	No. of women	Percentage	
23-30	21	46.67	31	62.2	0.3
31-35	15	51.11	14	37.7	
36-40	9	2.22	0	0	
Total	45	100	45	100	
Mean ± SD	31.7 ± 4.7		30.8± 2.9		

Table 2 : Distribution Of Study Population According To Increase In Endometrial Thickness After Injection G-CSF.

Change in ET after G-CSF	Group A		Group B		P-value
	Number of Women	Percentage	Number of Women	Percentage	
<1mm	3	6.6	1	2.2	0.0006
1-2mm	4	8.88	3	6.6	
>2mm	38	84.4	41	91.11	
Total	45	100	45	100	
Mean ± SD	0.74± 0.27		0.98± 0.36		



In above table we found that mean endometrial thickness was increased in both group but in group B endometrial thickness increased significantly compared to group A after the G-CSF injection.

Table 3:distribution Of Study Population According To Change In Pattern Of Endometrium Before And After G-CSF Injection.

Pattern of endometrium		Group A (subcutaneous)		Group B (intrauterine injection)		P-value
		Number of Women	Percentage		Percentage	
Homogeneous pattern	ET Before inj. G-CSF (300mcg)	4	8.89	3	6.67	1
	ET after 48hr of injG-CSF (300mcg)	4	8.89	3	6.67	
Triple line (TL)	ET Before inj. G-CSF (300mcg)	41	91.11	42	93.33	1
	ET after 48hr of injG-CSF (300mcg)	41	91.11	42	93.33	

In above table we found that there was no significant change seen in Homogeneous and Tri-laminar pattern of endometrium after the G-CSF injection in both the group.

Table 4: Distribution Of Study Population According To Implantation Rate.

Implantation Rate	Group A		Group B		P-value
	No. of women	Percentage	No. of women	Percentage	
Positive	12	26.6	18	40.0	0.27
Negative	30	66.67	25	55.56	
Total	45	100.00	45	100.00	

In above table we found that rate of implantation in group A was 26.6% and for group B it was 40%.

DISCUSSION
The study compared the effects of subcutaneous versus intrauterine administration of Granulocyte Colony-Stimulating Factor (G-CSF) on endometrial thickness, pattern , vascularity , and pregnancy outcome in women with persistently thin endometrium undergoing frozen-thawed embryo transfer (FET) cycles. Thin endometrium is a well-

documented factor associated with poor implantation and lower pregnancy rates in assisted reproductive technology (ART), particularly in FET cycles, where the endometrial environment plays a critical role in embryo implantation.

In present study patients with thin endometrium (<7 mm after maximum treatment) were allocated in two groups and G-CSF injection was given subcutaneously in Group A and intrauterine infusion in Group B. Increase in endometrial thickness was noted in both groups and patients were stratified according to increase in endometrial thickness.

Our findings indicate that both routes of G-CSF administration led to a statistically significant improvement in endometrial thickness. However, the intrauterine route showed a greater mean increase in endometrial thickness (0.98 + 0.36 mm) as compared to the subcutaneous route (0.74 + 0.27 mm), with a highly significant p-value of 0.0006. This supports earlier evidence that G-CSF may exert a more potent local effect when delivered directly to the uterine cavity, possibly due to its localized action on endometrial stromal cells and enhancement of angiogenesis and proliferation.

In present study the mean age for group A was 31.7 years and for group B it was 30.8 years. This data was similar to others studies like Singal S et al[7] who conducted a study on two group subcutaneous G-CSF and intrauterine G-CSF. They found that mean age of the participants was 33.3 ± 5.12 years. Baseline characteristics of patients showed non-significant difference.

We found that 91.11% of women in Group B experienced an increase in endometrial thickness of more than 2 mm after inj G-CSF intrauterine infusion, compared to 84.4% in Group A after inj G-CSF subcutaneously. Their was increase in endometrial thickness in both groups but in Group B endometrial thickness increased significantly more as compare to group A after the inj G-CSF. Both groups showed a trend toward trilaminar endometrial pattern post-treatment, no significant change in the pattern was observed, indicating that while G-CSF improves thickness, it may not consistently alter echogenicity or layering of the endometrium.

Our results of increase in endometrial thickness were consistent with results of study done by Gleicher et al[8], Singal S et al[7], Mishra VV et al[9], Kunicki et al[10], Barad et al[11] and Bin Xu et al[6]

The study also demonstrated higher implantation (40% in Group B vs. 26.6% in Group A), chemical pregnancy (44.4% vs. 33.3%), and clinical pregnancy rates (42.3% vs. 30.1%) in the intrauterine group, suggesting a possible improvement in endometrial receptivity beyond mere morphological changes. However, the difference in implantation and clinical pregnancy rates did not reach statistical significance, potentially due to the limited sample size. These trends, nonetheless, are clinically relevant and align with previous studies suggesting intrauterine G-CSF may enhance endometrial receptivity and subsequent pregnancy outcomes.

In the study of Xu and colleagues[6], significantly higher rates of implantation and clinical pregnancy were observed in their intervention group compared to their control group; they believed that increased clinical pregnancy rate is due to increased endometrial thickness.

Our results are consistent with those reported in previous studies such as Gleicher et al[8] and Barad et al[11] which suggested promising role of intrauterine G-CSF in improving outcome in women with recurrent implantation failure and thin endometrium.

Several mechanisms have been proposed for the beneficial effect of G-CSF on the endometrium. G-CSF is believed to promote the recruitment and differentiation of endometrial stem/progenitor cells, enhance angiogenesis, modulate immune tolerance at the maternal-fetal interface, and support trophoblastic invasion all critical for successful implantation. The localized administration through the intrauterine route could potentiate these effects by ensuring direct endometrial bioavailability and minimizing systemic dispersion.

Strength And Limitation Of Study:

One of the strength of our study is standardized protocol of endometrial evaluation by the same clinician on same ultrasound machine reduces bias.

However, variation in protocols , dosage and patient selection criteria across studies make it difficult to generalize these results .The sample size ,while adequate to detect differences in endometrial thickness , may not powered enough to detect significant differences in clinical outcome like implantation and pregnancy rates. Also, long term follow up data on live birth rate and neonatal outcome were not included.

CONCLUSION

Our study showed that to treat thin endometrium with intra-uterine route of G-CSF was more effective than subcutaneous route. Intrauterine route offers many advantages such as less invasive procedure , cost effective , easy to perform and no complication rate.We concluded that G-CSF infusion increases endometrial thickness, clinical pregnancy rate and lowers cycle cancellation rate. This will help in reducing financial and psychological burden on patient, and decrease their repeated hospital visits. The limitation of this study less number of patients and it is a single center study therefore in future we require more number of studies and randomised control trials to formulate an appropriate treatment protocol in patients with thin endometrium.

REFERENCES

1. Gardner DK, Weissman A, Howles CM, Shoham Z (2012) Textbook of Assisted Reproductive Techniques. 4th Ed. Clinical Perspectives. CRC press 464.
2. Lebovitz O, Orvieto R. Treating patients with “thin” endometrium - an ongoing challenge. GynecolEndocrinol. (2014) 30:409– 14.
3. https://www.medicalnewstoday.com/articles/assisted-reproductive-technology#definition
4. https://www.tandfonline.com/doi/abs/10.1080/09513590.2020.1740918?journalCode=igye20
5. Zhao J, Zhang Q, Wang Y, Li Y (2014) Endometrial pattern, thickness and growth in predicting pregnancy outcome following 3319 IVF cycle. ReprodBioMed Online 29(3):291–298.
6. Xu B, Zhang Q, Hao J, Xu D, Li Y. Two protocols to treat thin endometrium with granulocyte colony-stimulating factor during frozen embryo transfer cycles. Reprod Biomed Online. (2015) 30:349–58.
7. Singal S, Sharma R, Ahuja N. GCSF in patients with thin endometrium − subcutaneous or intrauterine?.FertilSci Res 2020;7:43-8.
8. Gleicher N., Vidali A., Barad D.H. Successful treatment of unresponsive thin endometrium // Fertil. Steril. 2011. Vol. 95. No. 6. P. 2123.e13–2123.e2.123E17.
9. Mishra V V, Choudhary S, Sharma U, Aggarwal R et al. Effects of Granulocyte Colony-Stimulating Factor (GCSF) on Persistent Thin Endometrium in Frozen Embryo Transfer (FET) Cycles. The Journal of Obstetrics and Gynecology of India (September–October 2016) 66(S1):S407–S41.
10. Kunicki M, Łukaszuk K, Woclawek-Potocka I, Liss J, Kulwikowska P, et al. (2014) Evaluation of granulocyte colony-stimulating factor effects on treatment-resistant thin endometrium in women undergoing in vitro fertilization. BioMed Res Int 913235.
11. Barad DH, Yu Y, Kushnir VA, Shohat-Tal A, Lazzaroni E, Lee HJ, et al. A randomized clinical trial of endometrial perfusion with granulocyte colonystimulating factor in in vitro fertilization cycles: impact on endometrial thickness and clinical pregnancy rates. FertilSteril 2014; 101:710-715.